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Response of Foetal and Maternal Spleens of Albino Rat to the Electromagnetic Field. Histological , Histochemical and Ultrastructural Studies.

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Abstract

Exposure of pregnant rats to electromagnetic field (EMF) of 50 Hz and 2 millitesla (2 mT), showed many deleterious changes in the spleen of these rats and their foetuses. Many histological , histochemical and ultrastructural changes were observed in foetal and maternal spleen. Altered polysaccharides, protein content and collagenous bundles were detected in spleen of pregnant rats and their foetuses. Foetal spleen appeared more sensitive to the electromagnetic field than the maternal spleen. Also mitochondria of WBCs were highly sensitive to these fields than Golgi apparatus.

Key words: Rats, Electromagnetic Field, Spleen, Foetuses.

Introduction

In today's world ,although technologic developments bring social and economic benefits to large sections of society , the health consequences of these developments are however difficult to predict and manage. With rapid advances in the electromagnetic fields (EMFs) technologies and communication , children are increasingly exposed to EMFs at earlier ages. Consistent epidemiologic evidence of association between childhood leukemia and exposure to extremely low frequency magnetic fields had led to their classification by the International Agency for Research on Cancer as a possible human carcinogen [Kheifets *et al.* , 2005]. The potential hazards of imposed magnetic fields and the interaction of magnetic field with cells, tissues and organs were studied by many authors [Gorczynska and Wegrzynowicz, 1991 ; Morelli *et al.* , 2005 and Eid & Al-Dossary , 2007].

A significant clustering of the distribution of intramembrane proteins post-exposure to EMF was observed by Morelli *et al.* (2005), who reported that EMF affects membrane associated enzymes activities. According to Gorczynska and Wegrzynowicz (1991), weak magnetic and electromagnetic fields might modify biochemical and physiological processes. They also added that paramagnetic properties of iron-storing organs such as liver , spleen and bone marrow make these organs likely to be affected by magnetic fields. Since these organs are intimately involved in haemopoietic and haemostatic balance , exposure to magnetic fields could be a possible biohazard. They noticed serious disturbances in mitochondria, endoplasmic reticulum and ribosomes in hepatocytes after exposure to 10^{-3} and 10^{-2} T 1 hr / daily.

Ryan *et al.* (2000) reported that exposure to EMFs increased cancer risk and they added that EMFs had the ability to impair reproductive processes (reduce fertility and / or alter foetal development). Laboratory studies on foetal development in mice and rats indicated increase in malformations after exposure to EMFs [Frolen *et al.*, 1993 ; Kheifets *et al.*, 2005 ; Parivar *et al.* , 2006 and Komazaki & Takano, 2007].

According to Kheifets *et al.* (2005) , environmental EMF exposures can be particularly harmful to children because of their special vulnerability during periods of development before and after birth. They added that children are more sensitive to EMFs because they have a longer lifetime of exposure than adults , and from a

physiological point of view , they have a developing nervous system , their brain tissue is more conductive than that of adults because it has a higher water content and ion concentration.

Male mice exposed to 2 mT , 50 Hz showed many pathological changes in RBCs and spleen tissue. Erythrocytes showed distortion , contraction and irregular pear-shape. Internal haemorrhage in the liver, spleen and testis with increased number of megakaryocytes and hyaline degeneration in the spleen were observed by .EL-Dahshan [2006].

The spleen was chosen for this study because it plays an important role in haemopoietic and haemostatic balance as well as in the body immunity.

Material and Methods

A. Animal model:

This work was carried out on Swiss albino rats,20 pregnant females(160 average weight)and 10 males kept for fertilization , each weighs about 160 grams. Animals were housed in plastic cages. They were kept under normal room conditions of temperature , humidity and normal light cycle and freely supplied with food and water.

B. Experimental design:

Female rats were categorized into the following groups :

Group (1): 10 pregnant rats were kept under normal conditions unexposed to electromagnetic fields.

Group (2) : 10 pregnant rats were exposed to alternating electromag-netic fields (50 Hz) with flux density 2 mT , 8 hours day after day , for 10 days before pregnancy then 20 days , day after day ,during pregnancy period(the total days of exposure were15 days), then they were sacrificed.

For alternating electromagnetic fields induction , exposure chamber was designed to obtain alternating current electromagnetic fields of 50 Hz frequency within it where the rats were placed between the poles of the electromagnet in a well ventilated plastic container which maintained a constant environmental temperature.

C. Histological and histochemical techniques:

Fresh small pieces of spleen specimens were fixed in 10 % neutral buffered formalin solution & Carnoy's Fluid for histological and histochemical studies. Paraffin sections were prepared 2 µm thickness and stained with Harris haematoxylin and eosin [Bancroft and Gamble, 2002]. Polysaccharides were detected by PAS (periodic acid Schiff) method [Hotchkiss , 1948]. Proteins were detected by mercuric bromophenol blue method [Mazia *et al.*, 1953]. Collagen was detected by Mallory trichrome stain [Pearse , 1977].

D. Electron microscopy technique:

E. Ultrastructural studies have been conducted on specimens prepared as follows: fresh spleen specimens were removed from all rat groups , cut into small pieces (0.5 – 1 mm) and immediately fixed in cold 4 % glutaraldehyde in 0.2 M cacodylate buffer (pH = 7.2) , and kept for 4 hr at 4° C [Sabatini *et al.*, 1963]. After washing in the same buffer specimens they were post-fixed in 1 % osmium tetroxide in cacodylate buffer (pH=7.2) then embedded in plastic resin [Robenson *et al.*, 1987]. Semithin sections (1 µm in thickness) were cut using ultramicrotome , mounted on glass slides and stained with toluidune blue for light microscopic examination.

Ultra-thin sections were also cut , mounted on coated copper grids , stained with uranyl acetate and lead citrate [Echlin , 1964] for transmission electron microscope examination (Jeol- JEM- 100 C X II – Transmission electron microscope King Faisal University, Dammam K.S.A) Negative Kodak film (Kodak film – verichrome pan – black & white – negative film – VP120) were used .

Results

Spleen structure of the control pregnant rat is shown in Fig. (1). Many degenerative changes were observed in the spleen of the pregnant rats post-exposure to

low frequency electromagnetic field (ELF-EMF) (Figs. 2-4). These changes include: thickened trabeculae with dilated and congested trabecular veins and arteries , increased megakaryocytes , appearance of many degenerated areas which contained debris of degenerated cells and many immature RBCs with increased signs of pyknosis. Thickened walls of the central arterioles with consequent narrow lumina were detected. These changes were more pronounced in the spleen of foetuses from exposed mothers (Fig. 14).

Increased signs of pyknosis and karyolysis were observed in the cells of the white pulps which were ill defined , The walls of central arterioles were highly thickened and most nuclei of the tunica intima and tunica media disappeared ,several megakaryocytes were also noticed. White pulps were invaded by RBCs. Many immature RBCs were detected and pools of RBCs occupied the degenerated areas spleen control.

Polysaccharides distribution in the spleen was observed in control pregnant rat in Fig. (5) and in those exposed to EMF (Figs. 6 - 7). Moderate increase in the polysaccharides stainability was observed in the thickened central arterioles in the spleen of pregnant rats exposed to EMF as compared with the control ones. Increased erythropoiesis was accompanied by dense stainability in RBCs. Dense stainability for polysaccharides was also noted in the trabeculae which appeared thickened and elongated.

Normal distribution of polysaccharides in the spleen of the control embryo was noted (Fig. 15). Mild stainability was observed in the capsule and RBCs in the red pulps, while WBCs appeared less stained.

Increased immature RBCs and megakaryocytes in spleen foetuses taken from exposed pregnant rats showed dense reaction for polysaccharides (Figs. 16 & 17) Increased RBCs which invaded the white pulps and those found in the congested trabecular veins were densely stained. Poor stainability was observed in the WBCs and the capsule.

Increased erythropoiesis was accompanied by increased protein content in the spleen of exposed pregnant rats (Figs. 9 & 10),as compared with control (Fig. 8). Thickened trabeculae and walls of the central arterioles also showed increased content of protein as compared with control .Generally, WBCs appeared poorly stained. In spleen of foetuses taken from exposed pregnant rats there wass a dense reaction for total proteins in increased RBCs (Fig. 19)as compared with control (Fig. 18) , but trabeculae and white pulps showed less stainability in the spleen of control foetuses and those taken from exposed pregnant rats.

Fig. (11) shows mild stainability for collagen bundles in the capsule , trabeculae and walls of blood vessels of the spleen of the control pregnant rats. Increased stainability for collagen fibres was observed in the thickened walls of trabeculae and walls of blood vessels of the spleen of the exposed pregnant rat , thin bundles were observed in the red pulps (Fig. 12) , but less stainability was observed in between red and white pulps. In spleen of control foetuses thin bundles of collagen were observed in the trabeculae as well as white and red pulps (Fig. 20). Thickened walls of trabeculae and their veins of spleen of foetuses taken from exposed mothers showed a slight increase in these bundles (Fig. 21) , but they were less stained in the red and white pulps.

The ultrastructural study of EMF exposed maternal and foetal spleen showed that lymphocytes in the white pulps were highly affected. Highly elongated nucleus with the reduction of the mitochondrial cristae and ribosomes with ruptured mitochondrial membranes were shown , while Golgi apparatus appeared more resistant to EMF. Disintegrated chromatin with increased number of cytoplasmic vacuoles were noted (Figs. 25 & 26) in comparison to control (Figs. 22 , 23 , 24). No detectable differences were observed in foetal and maternal lymphocytes of the spleen of control groups.

Foetal lymphocytes appeared more sensitive to EMF , their nuclei contained condensed or disintegrated chromatin with highly affected mitochondria which lost their cristae and showing ruptured mitochondrial membranes (Fig. 27). Plasma cells of the spleen of exposed pregnant rats showed a decrease in their cytoplasmic

density and contained reduced ribosomes with dilated cisternae of rER and condensed chromatin (Fig. 30) as compared with the control (Fig, 28). Embryonic plasma cells of the control group (Fig. 29) showed a decrase in the cytoplasmic density than that showed by the maternal ones (Fig. 28). Plasma cells of spleen of foetuses taken from exposed mothers accepted decreased cytoplasmic density (Fig. 31), with highly reduced ribosomes and disturbed rER and mitochondria. Megakaryocytes in the red pulp nearly lost their cytoplasmic organoids and chromatin (Fig. 32), while monocyte in the white pulp of the exposed pregnant rat showed highly affected mitochondria , which lost their cristae (Fig. 34).

RBCs in both exposed pregnant rats and their foetuses showed many pathological changes (Fig. 32 & 33). RBCs were distorted and fragmented and they contained disintegrated hemoglobin with lots of hemosiderin granules.

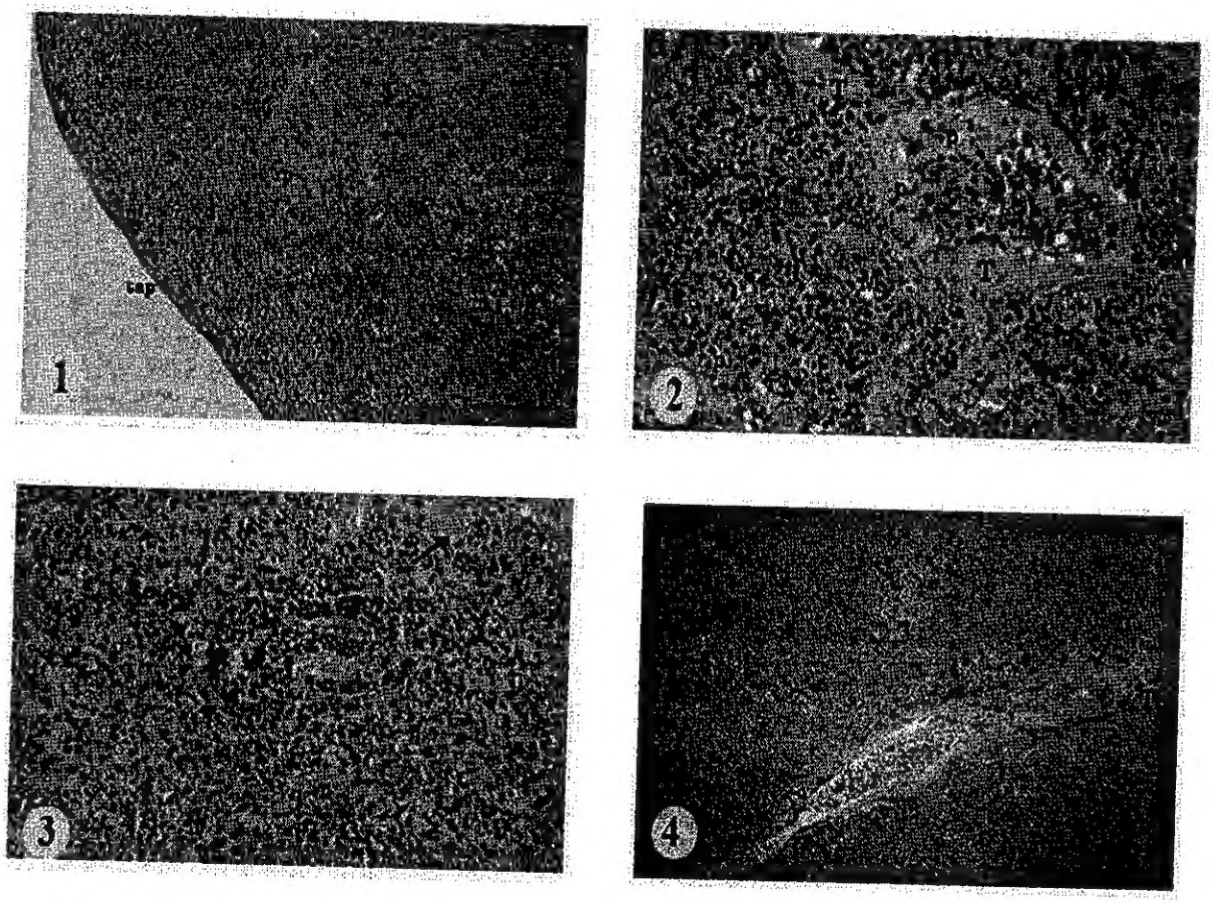


Fig. (1) Photomicrograph of section in spleen of control pregnant rat showing well developed structure which consists of : the white pulps (**wp**) with an eccentric central arterioles. These pulps are surrounded by the red pulps (**rp**). The spleen is covered with a thin fibrous capsule (**cap**) with many supporting trabeculae (**T**).

(H&E x 200).

Figs. (2 – 4) spleen of exposed pregnant rats stained with H&E.

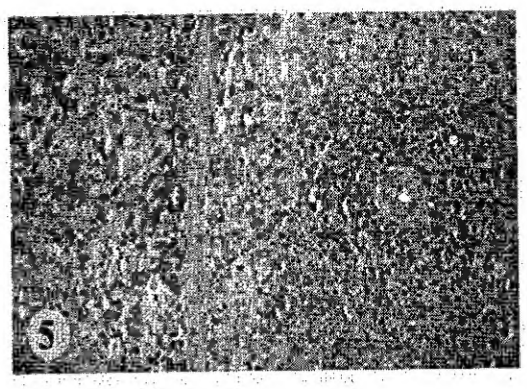
Fig. (2) Photomicrograph of section of spleen showing highly thickened trabeculae (**T**) which are invaded by lots of blood corpuscles , many megakaryocytes (**M**) [platelets forming cells] , many degenerated areas which contain debris of dead cells, many immature RBCs with lots of pyknotic cells (**p**).

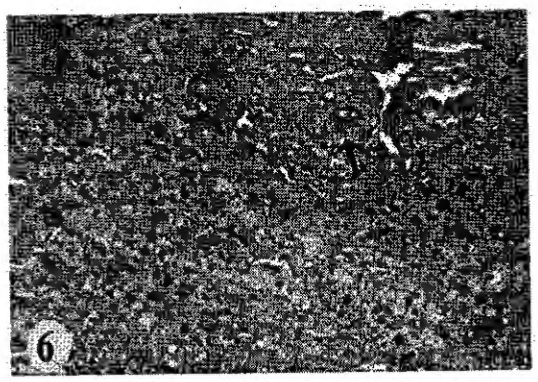
Fig. (3) Photomicrograph of section in spleen revealing highly disturbed lymphocytes in the ill defined white pulps with thickened wall of the central arteriole and presence of giant cells (**arrow**).

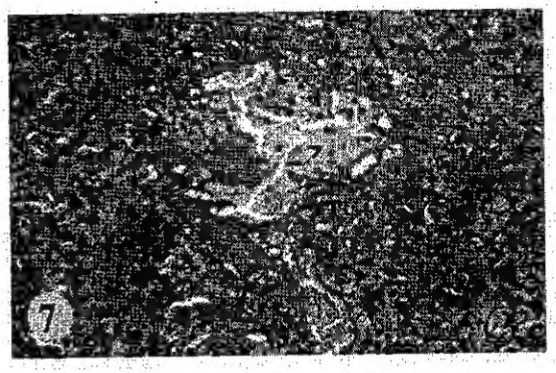
Fig. (4) Photomicrograph of section in spleen showing narrowed lumen of the thickened wall of central arterioles (>) and narrowed lumen in the thickened wall splenic artery (**arrow**), highly elongated and congested trabecular vein (**Tv**) in the higly elongated trabecula (**T**) with increased haemopoiesis in the red pulp.

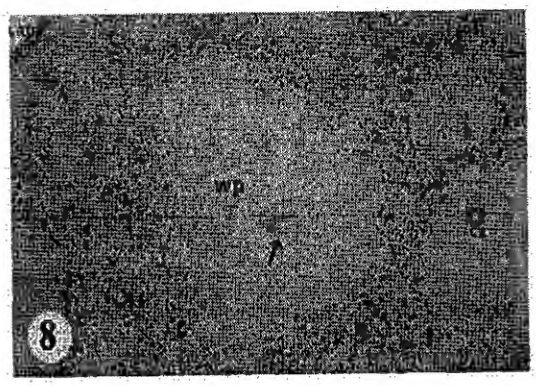
Figs. 2 - 3 (H&E x 400).

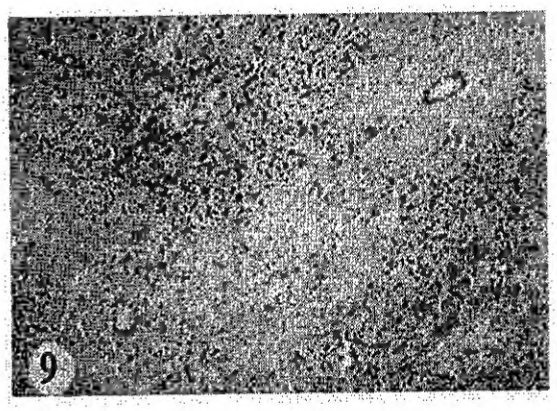
Fig. 4 (H&E x 200).











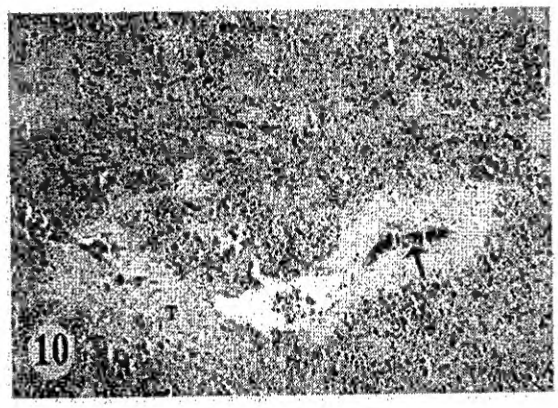


Fig. (5) Photomicrograph of section in spleen showing polysaccharides in the spleen of control pregnant rat.
Notice : Intense stainability in the RBCs , moderate stainability in the trabecula and the tunica media of the central arteriole but less stainability is observed in the WBCs. **(PAS x 400).**

Figs. (6 & 7) Photomicrographs of sections in spleen revealing polysaccharides in the spleen of exposed pregnant rats.

Fig (6) Photomicrograph of section of spleen showing moderate increase in the stainability in the thickened central arteriole (**arrow**) and dense reaction in the RBCs in the red pulp.

Figs. (7) Photomicrograph of section of spleen showing an increase in the stainability in the haemolysed blood corpuscles in the highly dilated and congested trabecular vein (**arrow**), while some areas inside it appear faintly stained (>) with dense reaction in the increased RBCs in the red pulp. Moderate stainability is observed in the highly thickened and elongated trabeculae (**T**). **Figs. 6 & 7 (PAS x 400).**

Fig. (8) Photomicrograph of section in spleen showing normal distribution of total proteins in the spleen of control pregnant rat. Dense stainability was observed in RBCs of the red pulp with less stainability in the wall of the central arteriole (**ca**), the trabeculae (<) and the capsule (**cap**) but weak stainability is observed in the cells of the white pulp (**wp**). **(Mercuric bromophenol blue x 200).**

Figs. (9 & 10) Photomicrographs of sections of spleen showing total proteins in exposed pregnant rats stained with Mercuric bromophenol blue.

Increased erythropoiesis is accompanied by increased stainability in RBCs in the red pulp (**rp**) & white pulp (**wp**) , inside trabecular vein (**arrow**) and those invaded the outer sheath of the white pulp. Enlarged trabeculae (**T**) show less stainability and so lymphocytes , but mild stainability could be observed in the walls of the central arterioles.
(Mercuric bromophenol blue x 200).

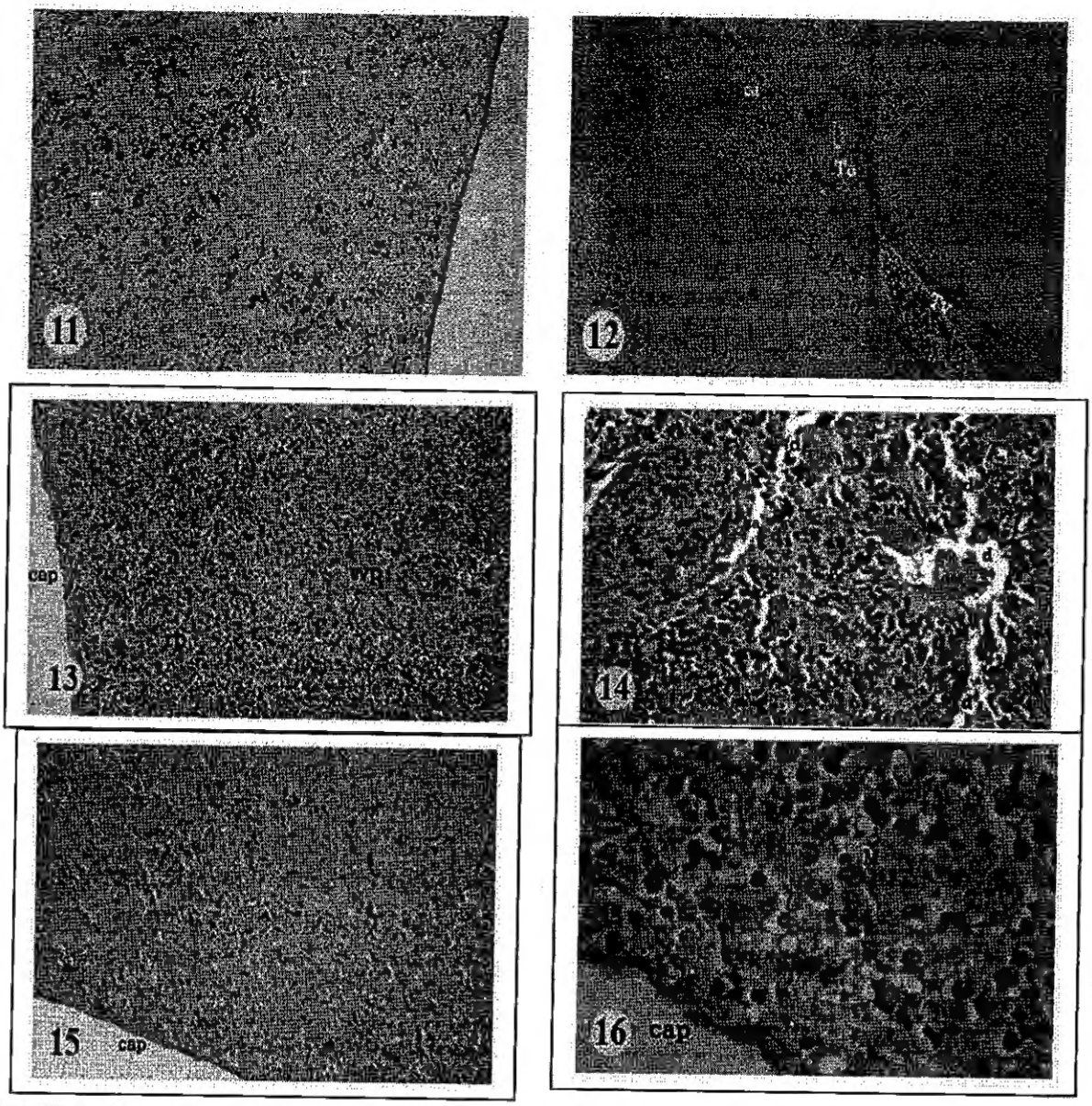


Fig. (11) Photomicrograph of section in spleen showing normal distribution of collagenous bundles in the spleen of control pregnant rat. Mild staiñability for collagen is noticed is the capsule (**cap**), trabeculae (**T**), walls of blood vessels and thin bundles in between white and red pulps **(Mallory trichrome stain x 200).**

Fig. (12) Photomicrograph of section in spleen revealing distribution of collagenous bundles in the spleen of exposed pregnant rats. Increased stainability is observed in the thickened trabeculae and in the walls of highly congested trabecular veln (**Tv**) and the thickened wall of the trabecular artery (**Ta**) and so the wall of the central arteriole (**ca**). **(Mallory trichrome stain x 200).**

Fig. (13) Photomicrograph of section in spleen showing the normal structure of spleen of control embryo which consists of : white pulp (**wp**) , red pulp (**rp**) , they are encircled by the capsule (**cap**) with increased signs of haemopoiesis. **(H&E x 200).**

Fig. (14) Photomicrograph of section in spleen showing lots of degenerated areas (**d**) in the spleen of foetuses taken from the exposed rat, some of them contained debris of the dead cells (**de**) , many pyknotic cells (**p**) , others are karyolytic (**ka**) , highly disturbed lymphocytes with vacuolated cytoplasm in the white pulp with disappearance of most nuclei of the cells of the wall of the central arteriole. Many megakaryocytes are also observed with many immature RBCs (nucleated). **(H&E x 200).**

Fig. (15) Photomicrograph of section in spleen showing normal distribution of polysaccharides in the spleen of control embryo. Mild stainability is detected in the capsule (**cap**) and RBCs in the red pulp with less stainability in the white pulp. **(PAS x 200).**

Figs. (16 & 17) Photomicrographs of sections in spleen revealing polysaccharides distribution in the spleen of foetus taken from the exposed rat.

Fig. (16) Photomicrograph of histological section of spleen showing fine granules in the capsule (**cap**) and in some RBCs. Megakaryocytes (M) show moderate reaction with poor stainability in WBCs. **(PAS x 400).**

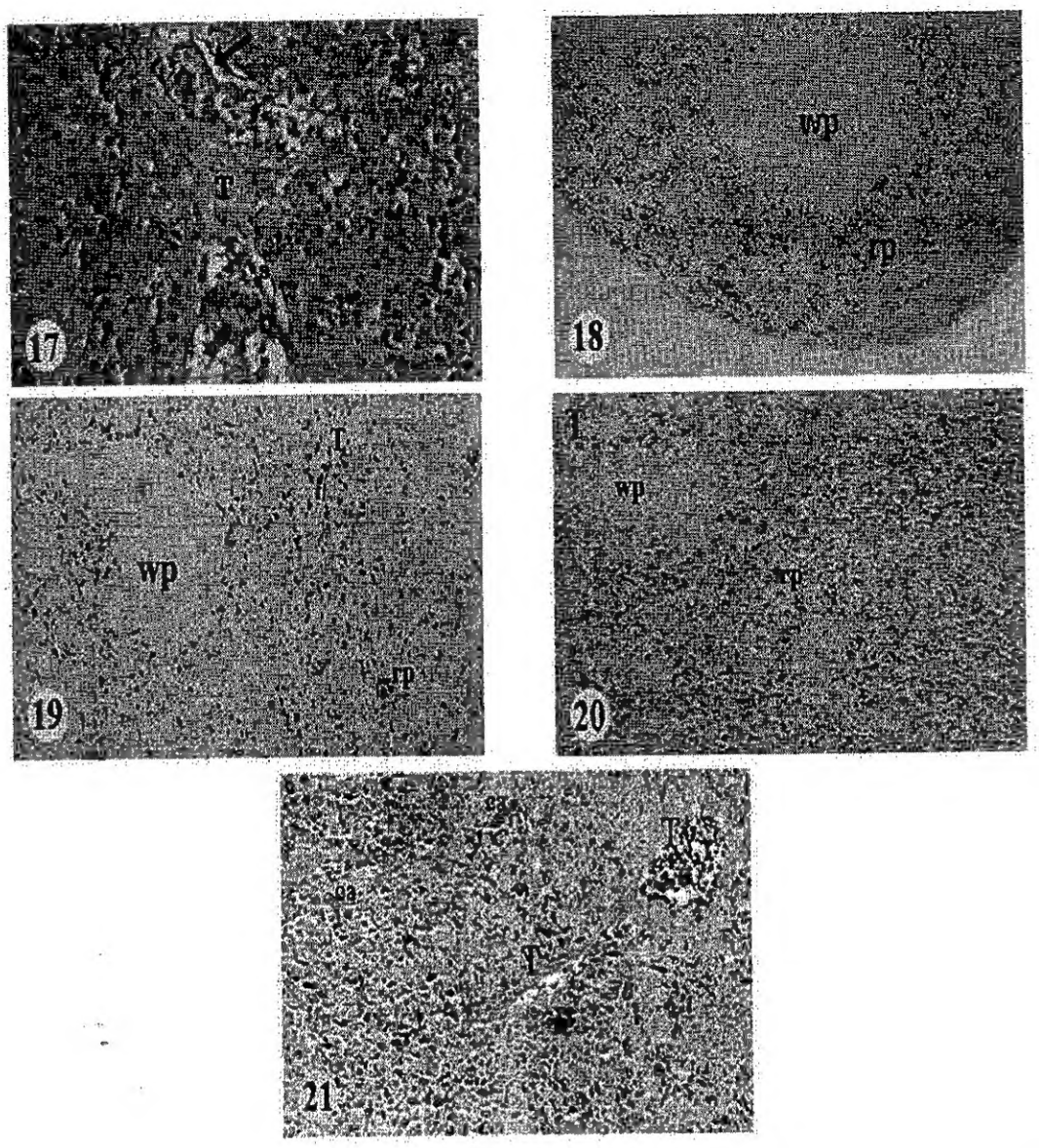


Fig. (17) Photomicrograph of section in spleen showing dense stainability in the dilated and congested trabecular vein (**s**), haemolysed RBCs in it show dense reaction (arrow), fine granules are observed in the remnant RBCs with less stainability in the trabeculae (**T**). Poor stainability could be detected in the WBCs.

Figs. 16 & 17 (PAS x 400).

Fig. (18) Photomicrograph of section in spleen showing the normal distribution of total proteins in the spleen of the control embryo. Moderate to dense reaction can be observed in RBCs in the red pulp (**rp**) with less reaction in the white pulp (**wp**).

(Mercuric bromophenol blue x 200).

Fig. (19) Photomicrograph of section in spleen showing dense reaction for total proteins in the increased RBCs in the red pulp (**rp**) of foetus taken from the exposed rat with poor stainability in the trabecula (**T**) and white pulps (**wp**).

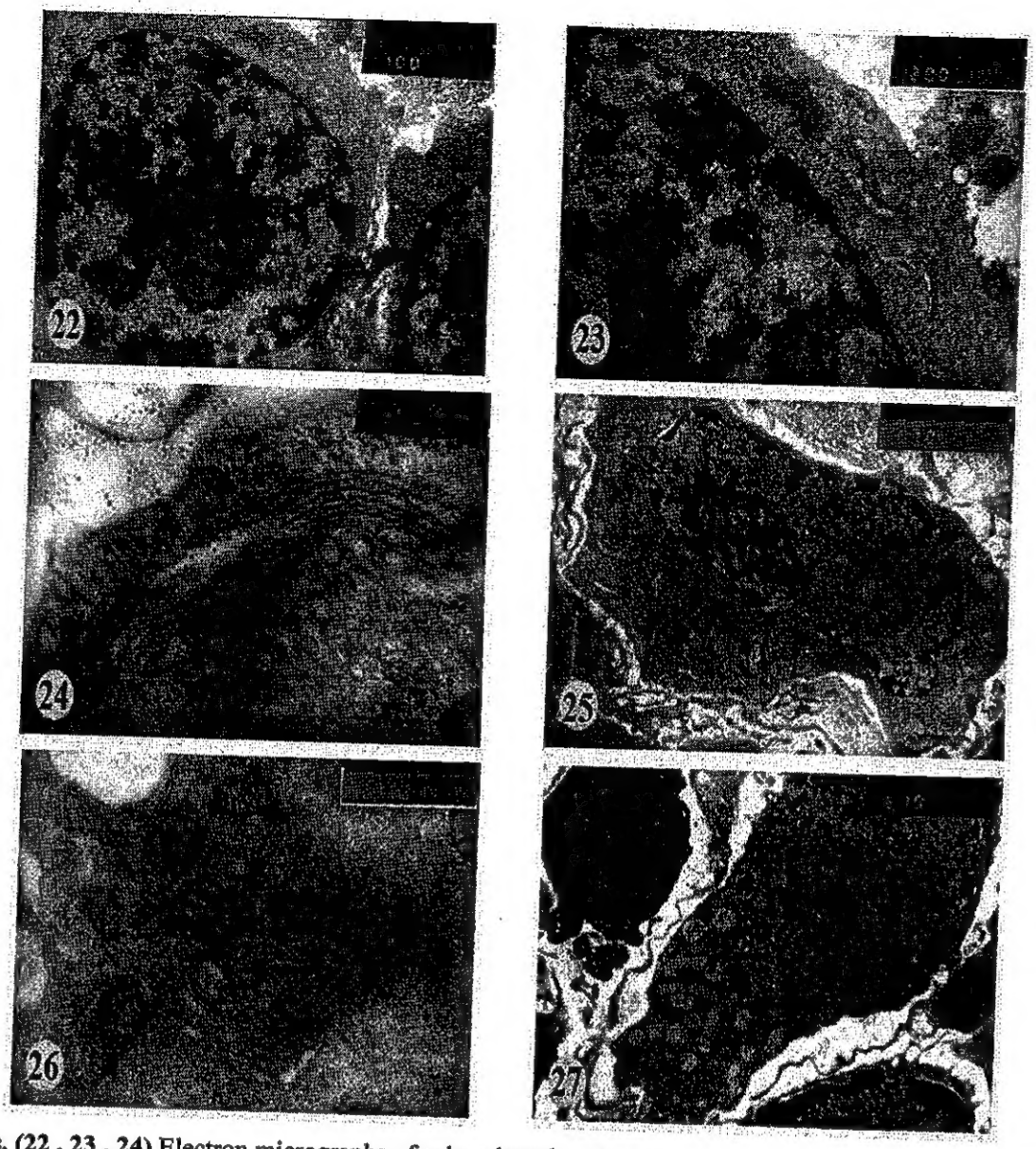
(Mercuric bromophenol blue x 200).

Fig. (20) Photomicrograph of section in spleen revealing normal distribution of collagenous bundles in the spleen of control embryo. Thin and few bundles are distributed in the trabeculae (**T**), white pulps (**wp**) and red pulps (**rp**).

(Mercuric bromophenol blue x 200).

Fig. (21) Photomicrograph of section of spleen showing slight increase of collagenous bundles in the walls of the trabeculae (**T**) and their veins (**Tv**) with less stainability in the red pulp and white pulps with thick layer in the thickened walls of central arterioles (**ca**) of foetus taken from the exposed rat.

(Mallory trichrome stain x 200).



Figs. (22 , 23 , 24) Electron micrographs of spleen lymphocytes taken from control pregnant rat showing : a large nucleus which is surrounded by scanty cytoplasm which contains few mitochondria, well developed Golgi apparatus and free polyribosomes. The nucleus has condensed chromatin which appears as coarse clumps.

22. (x 10000)
23. (x 20000)
24. (x 40000)

Figs. (25 & 26) Electron micrographs Showing highly elongated nucleus of lymphocyte taken from spleen of a pregnant rat. This nucleus contains marginal and fragmented chromatin. Some of mitochondria lost their cristae and have ruptured mitochondrial membranes, while Golgi apparatus appears more resistant.

25. (x 6700)
26. (x 2700)

Fig. (27) An electron micrograph Showing highly affected lymphocytes in the white pulp of the spleen of foetus taken from the exposed rat, some cells contained condensed chromatin others contained fragmented chromatin with highly disturbed cytoplasmic organoids. **(x 6700)**

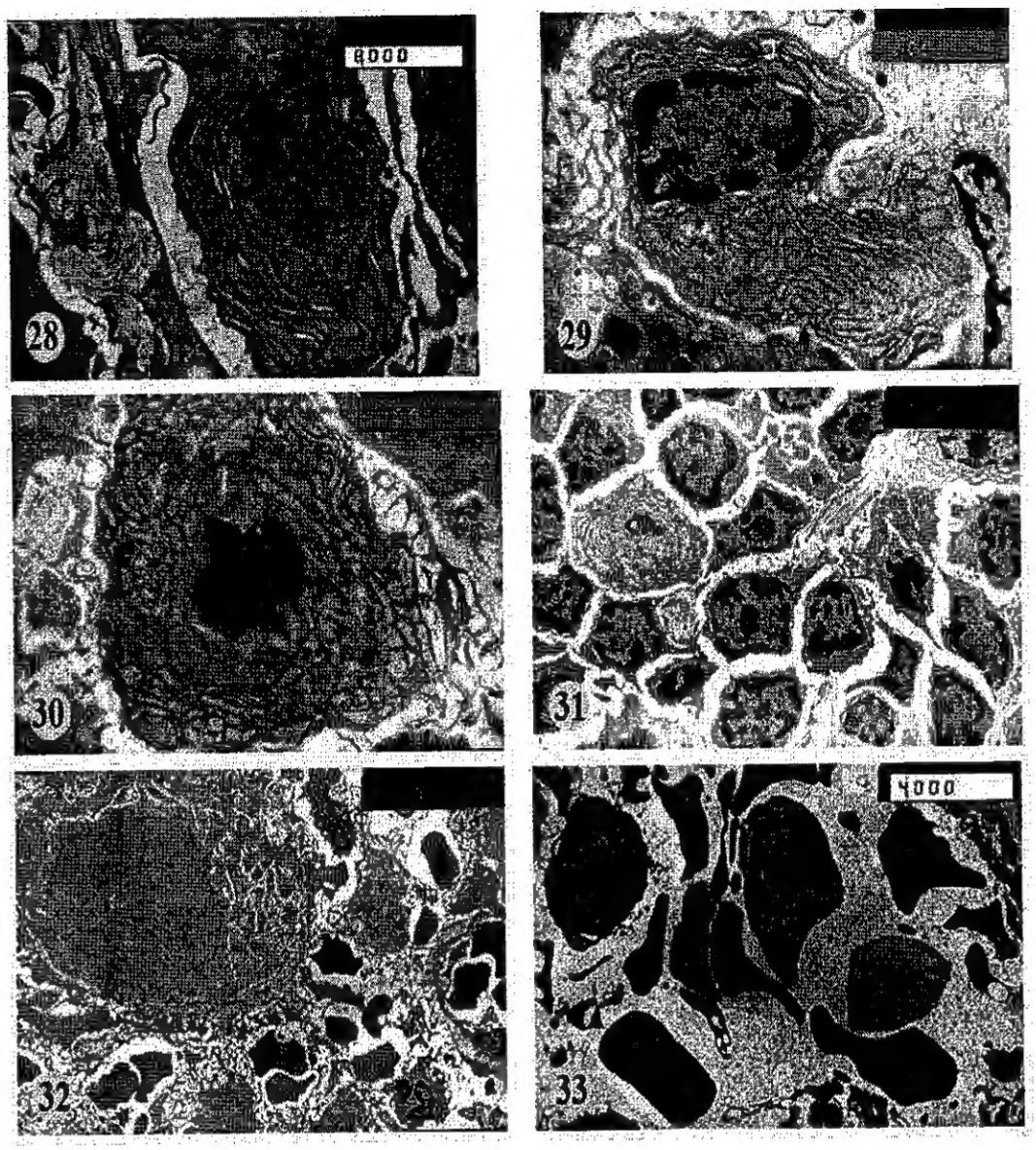


Fig. (28) An electron micrograph of the plasma cell in the white pulp of the control pregnant rat spleen showing : well developed rER , mitochondria , well developed Golgi apparatus and lots of ribosomes. **(x 6700)**

Fig. (29) An electron micrograph of the white pulp of the spleen of the control embryo showing : the plasma cell shows a decrease in the cytoplasmic density compared with that of the pregnant female rat. **(x 8000)**

Fig. (30) An electron micrograph showing highly affected plasma cell of an exposed pregnant rat which shows a decrease in the cytoplasmic density . Highly condensed chromatin is observed in the nucleus with undulating nuclear envelope and dilated cisternae of rER which show a decrease in the cytoplasmic density with ruptured cell membrane.

(x 10000)

Fig. (31) An electron micrograph of the plasma cell of spleen of a foetus taken from the exposed pregnant rat which appeared poorly stained with disintegrated chromatin. This cell was surrounded by lots of disturbed lymphocytes which show irregular distribution of chromatin. **(x 2700)**

Fig. (32) An electron micrograph showing highly affected megakaryocyte of the spleen of a foetus taken from the exposed pregnant rat. Nearly chromatin and cellular organoids can not be detected. Distorted and fragmented RBCs contain disintegrated hemoglobin and lots of hemosiderin granules. **(x 2000)**

Fig. (33) An electron micrograph showing distorted RBCs of an exposed pregnant rat. **(x 4000)**

Fig. (34) An electron micrograph showing highly affected monocyte in the white pulp of spleen of the exposed pregnant rat. Corrugated nuclear envelope is observed and some mitochondria lost their cristae . **(x 8000)**

Discussion

The Spleen was chosen for this study being the largest immune organ. The results of the present study show that exposure to electromagnetic field of 50 Hz & 2 mT resulted in lots of pathological changes in spleen of the pregnant rats and their foetuses. These changes were more drastic in spleen of exposed foetuses. In the pregnant rats these changes include : thickened trabeculae , dilated and congested trabecular veins and arteries , many degenerated areas with debris of degenerated cells , thickened walls of the central arterioles with narrowed lumina, lots of immature RBCs and condensed chromatin inside the distorted WBCs.

In foetuses, these changes were more pronounced , since lots of leucocytes contained condensed or fragmented chromatin with highly disturbed lymphocytes.

Increased megakaryocytes , thickened walls of the central arterioles contained enlarged nuclei in their cells with ill-defined white pulps which were invaded by RBCs.

Increased immature RBCs and lots of pools of RBCs which occupied the degenerated areas in the red pulps were also observed.

In agreement with the results of this study , EL-Kholy (1999) studied the effect of 50 Hz on the spleen of mice and he noticed cytoplasmic and nuclear degeneration and necrosis in WBCs. Also EL-Dahshan in 2006 exposed male mice to magnetic field of 50 Hz & 2 mT and she noticed highly affected hemoglobin with changes in the relative mobilites and the total number of protein bands as a result of appearance of new bands and disappearance of others. She also detected increased viscosity and conductivity of hemoglobin in exposed mice , contraction , anisocytosis , macrocytosis , polychromasia and irregular pear shaped RBCs. She noticed internal hemorrhage in spleen of exposed mice , increased number of megakaryocytes , hemosiderin granules , hyaline degeneration and enlarged spleen.

The noticeable sensitivity of spleen to EMF in this study may be due to paramagnetic properties of ion storing organs such as spleen.

According to Gorczynska and Wegrzynowicz (1991) , these paramagnetic properties in liver , spleen and bone marrow make these organs likely to be affected by electromagnetic fields , since these organs are intimately involved in haemopoietic and haemostatic balance and accordingly they added that exposure to EMFs could be a possible biohazard.

The present results indicated that foetuses are more sensitive to EMF than pregnant females. Kheifets *et al.* (2005) stated that this sensitivity may be due to the developing nervous system, their brain tissue is more conductive than that of adults because it has a higher water content and ion concentration. They also stated that

exposure to toxic agents such as EMFs and ionizing radiation may increase the risk of induction or progression of cancer and increased apoptosis and DNA damage during embryonic and childhood development.

Increased megakaryocytes which were observed in the present study drew the attention of many authors. Obukhan (1999) stated that the most sensitive indices of the blood system due to EMF exposure are disorders in megakaryocytes differentiation , polymorphism and disturbances in leucocytes ,whereas , these changes may be revealed as EMF markers to carry out an express-diagnostic tests.

According to Arafa *et al.* (2003), EMFs decreased splenocyte viability , WBCs count , as well as mitogens induced lymphocyte proliferation , L-carnitine, but not Q10 could ameliorate the adverse effect of EMF on the vast majority of the immune parameters , suggesting a possible immuno-protective role of L-carnitine.

Canseven *et al.* (2006) suggested that part of the immune system , the NK cell , can be suppressed by 50 Hz magnetic field. In exposed pregnant rats and their foetuses , increased content of polysaccharides was observed in the spleen tissue , especially in the increased RBCs in red pulps and congested blood vessels because they contain 10 % carbohydrates [Junqueira and Carneiro, 2003]. Thickened walls of trabeculae , central arterioles and trabecular blood vessels showed moderate increase in polysaccharides while degenerated areas appeared negatively stained. According to Casarette (1986) ; Zaghloul and Salem (2001) ; Koyu *et al.* (2005) and Manikonda (2007), these changes in polysaccharides may be due to failure of Golgi apparatus to synthesize carbohydrate or due to lytic enzymes released from ruptured lysosomes or due to hypoxia.

Furthermore, in exposed pregnant rats and their foetuses, increased erythropoiesis was accompanied by increased stainability in RBCs in the red & white pulps , inside trabecular vein and those invaded the outer sheath of white pulps with less stainability in trabeculae and lymphocytes and mild stainability in the walls of the central arterioles. Decreased protein content was observed in spleen of exposed foetuses with the exception of increased RBCs. According to Santoro *et al.* (1997), EMFs interfere with protein phosphorylation and modify the plasma membrane structure and interfere with the initiation of the signal cascade pathways. Decreased protein content observed in this study may be due to ruptured cellular organoids or to decreased polyribosomes. These results are in agreement with those of Khaki *et al.* (2006).

Decreased protein content was observed post-exposure to EMF by many authors [Howie , 1986 ; AL-Gahtani, 2006 and Eid & Al-Dossary , 2007].

Marked increase in the collagenous bundles was observed in spleen of exposed pregnant rats with slight increase in the embryonic spleen. This increased stainability was observed in the thickened trabeculae and thickened walls of blood vessels. In this respect , Khaki *et al.* (2006) stated that infoldings noticed in tissues post-exposure to EMF may be due to disturbed collagen content and loss in reticular fibres from the inner and outer non cellular layers.

Increased collagenous bundles in rats post-exposure to EMF was observed by Ottani *et al.* (1987). Oriented collagen and total protein content were observed in rats and mice exposed to EMF [Al-Gahtani , 2006 and Eid & Al-Dossary , 2007].

The haemopoietic organs like spleen being a storage of magnetic ions , particular ions seem to be more sensitive to the magnetic field being more influenced than the other organs [Gorczynska , 1987].

RBCs & WBCs in spleen of exposed pregnant rats and their foetuses appeared highly affected. Lymphocytes , plasma cells , monocytes and megakaryocytes contained disintegrated or condensed chromatin. Mitochondria in these cells lost some of their cristae with ruptured mitochondrial membranes , but Golgi apparatus appeared more resistant to EMF. Nuclei and cytoplasm of the embryonic cells appeared more sensitive to EMF.

Strong relationship between EMF exposure and increased incidence of certain childhood diseases was reported by many authors [Tynes and Haldorsen, 1997 ; Obukhan , 1999 ; Kheifets *et al.* , 2005 and Schuz *et al.* , 2007].

Many pathological changes were detected in lymphocytes post-exposure to EMF. These changes can modulate the immune parameters in different animals [Marino *et al.*, 2001 and Cho & Chung , 2003].

Megakaryocytes which produce blood platelets were highly affected by EMF. Obukhan (1999) stated that the most sensitive indices of blood system for EMF exposures are disorders in megakaryocytes differentiation and disturbances in the structure of leucocytes. Disturbed DNA and highly affected cisternae of rER as observed in this study and reduced ribosomes in leucocytes of exposed mother and their fetuses, may lead to disturbed immunity and reduced antigens synthesis. In 2000, Marino *et al.* concluded that exposure to power-frequency fields produced changes in the immune system that were both real and inconsistent.

In this study RBCs appeared highly sensitive to EMF. Many of authors studied the influence of EMF on erythrocytes. Gorczynska (1987) stated that the disturbance of ion metabolism in the body may be expressed to the presence of large granularity of hemosiderin in the spleen. He added that the decrease in ceruloplasmin activity in serum can disturb the ion transport in the body, and result in a considerable morphological changes in the spleen of exposed guinea pigs.

Conclusion

Results of the present study showed that exposure of pregnant rats to50 Hz and2 millitesla caused many dystrophic changes in the foetal and maternal spleens .

It is clear that splenic tissue of the pregnant rats is sensitive to electromagnetic fields, while embryonic spleen is highly sensitive to these fields. Mitochondria of WBCs are more sensitive to these fields than Golgi apparatus.

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استجابة الطحال فى أجنة الجرذان البيضاء وأمهاتها للمجال الكهرومغناطيسي

دراسة هستولوجية ، هستوكيميائية وخلوية دقيقة

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تضمن هذا البحث تأثير التعرض للمجال الكهرومغناطيسي ذو التردد 50 هرتز و كثافة فيض مغناطيسي 2 ملي تسلا على طحال أجنة الجرذان البيضاء و أمهاتها. ولقد لوحظ تأثر طحال أمهات الجرذان بهذا المجال بينما كان التأثر أكثر حده في طحال الأجنة. ولقد لوحظت تغيرات هستولوجية عديدة في طحال الأجنة و أمهاتها، كما حدث خلل في توزيع الخلايا اللمفية في مناطق اللب الأبيض لطحال الأجنة والتي كان من الصعب تحديدها كما انتشرت برك من خلايا الدم الحمراء بمناطق اللب الأحمر وظهر في بعض هذه الخلايا أنوية ، ولوحظ تحلل للهيموجلوبين في بعض هذه الخلايا وظهور حبيبات الهيموسيدرين في الخلايا الحمراء غير المنتظمة الشكل. كما لوحظ التضخم الشديد لجدر العوارض و الشرايين المركزية الموجودة بمناطق اللب الأبيض مع احتقان و تضخم أوردة و شرايين العوارض ،كما لوحظت مناطق تحطم خلوي عديدة. ولقد لوحظت تغيرات عديدة في محتوى الخلايا من السكريات العديدة والبروتينات و الألياف الكولاجينية في طحال الأمهات المعرضة وأجنتها. ولقد أوضح الفحص المجهري الدقيق تأثر الخلايا اللمفية والخلايا العملاقة والخلايا البلازمية وخلايا الدم البيضاء وحيدة النواة وخلايا الدم الحمراء سواءً في طحال الأجنة أو أمهاتها التي عرضت للـ EMF.